（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K022-M

Specification：48T(44 samples)/96T(92 samples)

Measuring instrument：Microplate reader (530-570 nm)

Detection range：1.35-62 U/mL

Elabscience® CuZn/Mn Superoxide Dismutase (CuZn-SOD/Mn-SOD) Activity Assay Kit (Hydroxylamine Method)

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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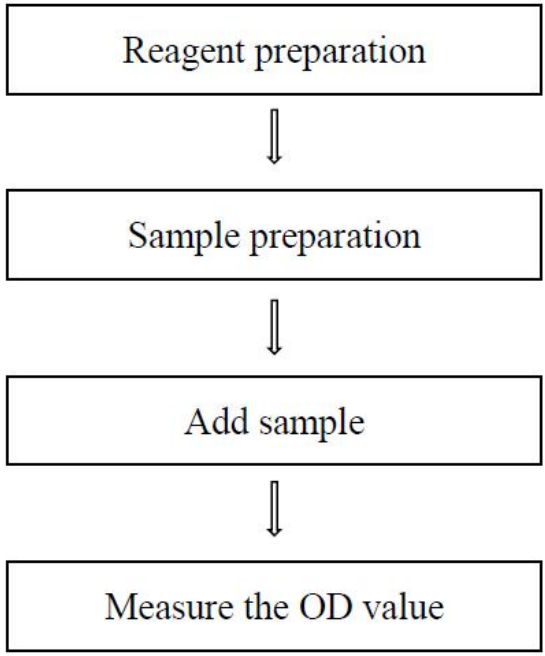
Assay summary

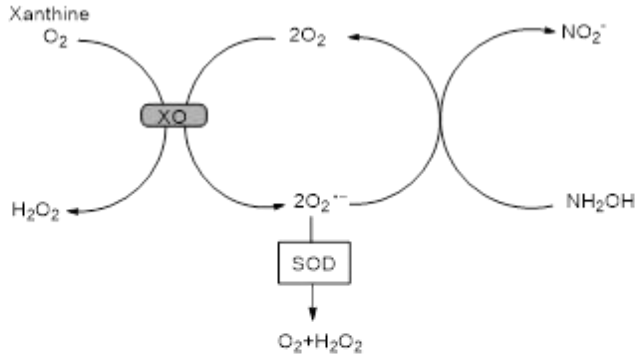


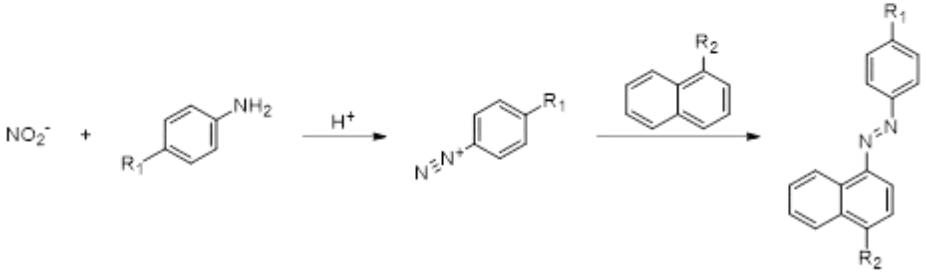
Intended use

This kit can be used to measure T-SOD, CuZn-SOD, Mn-SOD activity in serum, plasma, urine, cells, cell culture supernatant and tissue homogenate samples.

Detection principle

Superoxide anion (O2•-) produced by xanthine and xanthine oxidase system can oxidize hydroxylamine to form nitrite which appear purplish red after chromogenic reaction. The SOD in the sample has a specific inhibitory effect on superoxide anion (O2•-), can reduce the content of nitrite. The OD value is lower than control and the activity of SOD is calculated through the formula. There are two kinds of SOD (CuZn-SOD, Mn-SOD) in the cells of higher animals, and the sum of them is equal to the total SOD. The activity of Mn-SOD in the sample will lost after sample pretreatment, but the activity of CuZn-SOD will not.





Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	1.2 mL × 1 vial	1.2 mL × 1 vial	2-8℃, 12 months
Reagent 2	Nitrosogenic Agent	1.2 mL × 1 vial	1.2 mL × 1 vial	2-8℃, 12 months
Reagent 3	Substrate Solution	1.2 mL × 1 vial	1.2 mL × 1 vial	2-8℃, 12 months
Reagent 4	Enzyme Stock Solution	0.03 mL × 1 vial	0.06 mL × 1 vial	-20℃, 12 months
Reagent 5	Enzyme Diluent	1.2 mL × 1 vial	1.2 mL × 1 vial	2-8℃, 12 months
Reagent 6	Chromogenic Agent A	Powder × 1 vial	Powder × 1 vial	2-8℃, 12 months shading light
Reagent 7	Chromogenic Agent B	Powder × 1 vial	Powder × 1 vial	2-8℃, 12 months shading light
Reagent 8	Chromogenic Agent C	6 mL × 1 vial	6 mL × 1 vial	2-8℃, 12 months
Reagent 9	Extracting Solution	6 mL × 1 vial	12 mL × 1 vial	2-8℃, 12 months shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (530-570 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Keep enzyme stock solution on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of buffer working solution：

For each well, prepare 90 uL of buffer working solution (mix well 9 uL of buffer solution and 81 uL of double distilled water). Store at 2-8°C for 3 months.

③ The preparation of enzyme stock working solution：

Before testing, please prepare sufficient enzyme stock working solution according to the test wells. For example, prepare 100 μL of enzyme stock working solution (mix well 5 uL of enzyme stock solution and 95 uL of enzyme diluent). Store at 2-8°C for 3 days.

④ The preparation of chromogenic agent A application solution：

Dissolve one vial of chromogenic agent A with 70-80℃ double distilled water to a final volume of 9 mL. Store at 2-8℃ for 3 months protected from light.

⑤ The preparation of chromogenic agent B application solution：

Dissolve one vial of chromogenic agent B with double distilled water to a final volume of 9 mL. Store at 2-8℃ for 1 months protected from light.

⑥ The preparation of chromogenic agent：

For each well, prepare 200 uL of chromogenic agent (mix well 75 uL of chromogenic agent A application solution, 75uL of chromogenic agent B application solution and 50 uL of chromogenic agent C). The chromogenic agent should be prepared on spot. Store at 2-8°C for 1 month protected from light.

⑦ The preparation of enzyme working solution：

(Operate on ice) For each well, prepare 30 uL of enzyme working solution (mix well 10 uL of nitrosogenic agent, 10 uL of substrate solution and 10 uL of enzyme stock working solution). The enzyme working solution should be

prepared on spot, and it must be use out within 20 min.

⑧ The preparation of non-enzyme working solution:

For each well, prepare 30 uL of non-enzyme working solution (mix well 10 uL of nitrosogenic agent, 10 uL of substrate solution and 10 uL of enzyme diluent).

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Urine: Collect fresh urine and centrifuge at 10000 g for 15 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection. If not detected on the same day, the urine can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cells:

① Harvest the number of cells needed for each assay (initial recommendation 10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 10^6 cells in 300-500 μL normal saline (0.9% NaCl) with a

ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The optimal sampling volume are different for different species, the SOD also are different for different samples. It is recommended to take 2~3 samples to do a pre-experiment, diluting a series of diluent and determine the dilution factor when the SOD inhibition ratio is 15%~55% (the optimal inhibition ratio is the range of 25%~45%) before formal experiment.

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Mouse serum	5-10
Rat serum	6-15
Urine	2-3
Human hydrothorax	2-3
10% Mouse liver tissue homogenate	100-200
10% Mouse brain tissue homogenate	20-30
10% Mouse kidney tissue homogenate	50-120
10% Rat kidney tissue homogenate	50-120
HepG2 cells (5.21mgprot/mL)	15-25
Cell culture supernatant	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Determine optimal sampling volume of each sample before formal experiment. Calculate the inhibition ratio of serial sampling volume, and choose the optimal sampling volume when inhibition ratio in the range of 25%~45%.

② The optimal sampling volume are different for different species, the SOD also are different for different samples. So it is best to do a pre-test to determining optimal sampling volume for a new sample.

③ It is best to reserve 3 paralleled tubes with different sampling volumes in pre-test for determining the optimal sampling volume.

④ Adjust sampling volume: If inhibition ratio >55%, need to dilute the sample or decrease the sampling volume than take the test. If inhibition ratio < 15%, need to increase the sampling volume.

⑤ There should be no bubbles in the wells of the microplate when measuring the OD value.

⑥ The multichannel pipeter is recommended to shorten the time and reduce the error between wells.

Operating steps

Sample pretreatment

① Take 0.1 mL sample and add 0.1 mL extracting solution. Mix thoroughly with a vortex mixer for 1 min by vortex mixer and centrifuge at 3500 g for 15 min. Take supernatant for CuZn-SOD measurement.

② Take 0.1 mL normal saline and add 0.1 mL extracting solution. Mix thoroughly for 1 min with a vortex mixer and centrifuge at 3500 g for 15 min. Take supernatant as control of CuZn-SOD.

The measurement of samples

① Blank well of T-SOD: add 5 μL of double distilled water into the corresponding wells.

Control well of T-SOD: add 5 μL of double distilled water to the corresponding wells.

Sample well of T-SOD: 5 μL of sample to the corresponding wells.

Blank well of CuZn-SOD: add 5 μL of supernatant as control of CuZn-SOD into the corresponding wells.

Control well of CuZn-SOD: add 5 μL of supernatant as control of CuZn-SOD to the corresponding wells.

Sample well of CuZn-SOD: add 5 μL of supernatant for CuZn-SOD to the corresponding wells.

② Add 90 μL of buffer working solution to each well.

③ Add 30 μL of enzyme working solution to control well of T-SOD, sample well of T-SOD, control well of CuZn-SOD, sample well of CuZn-SOD.

Add 30 μL of non-enzyme working solution to blank well of T-SOD and blank well of CuZn-SOD.

④ Mix fully for 10 s with microplate reader and incubate at 37℃ for 50 min.

⑤ Add 180 μL of chromogenic agent to each well.

⑥ Mix fully for 10 s with microplate reader and stand for 10 min at room temperature. Measure the OD values of each well at 550 nm with microplate reader.

Calculation

The sample:

1. **Serum (plasma), cell culture medium and other liquid samples:**

Definition: When SOD inhibition ratio in 1 mL reaction system reach 50%, the corresponding enzyme level is 1 SOD activity unit (U).

$$\text{T-SOD activity (U/mL)} = i_1 \div 50\% \times \frac{V_1}{V_2} \times f$$

$$\text{CuZn-SOD activity (U/mL)} = i_2 \div 50\% \times \frac{V_1}{V_2} \times f$$

2. **Tissue and cell samples:**

Definition: When SOD inhibition ratio of 1 mg of tissue protein in 1 mL reaction system reach 50%, the corresponding enzyme level is 1 SOD activity unit (U)

$$\text{T-SOD activity (U/mL)} = i_1 \div 50\% \times \frac{V_1}{V_2} \times f \div C_{pr}$$

$$\text{CuZn-SOD activity (U/mL)} = i_2 \div 50\% \times \frac{V_1}{V_2} \times f \div C_{pr}$$

Mn-SOD activity =T-SOD activity -CuZn-SOD activity

[Note]

i_1: Inhibition ratio of T-SOD.

$$i_1 (\%) = \frac{(A_1-A_3)-(A_2-A_3)}{A_1-A_3} \times 100\% = \frac{A_1-A_2}{A_1-A_3} \times 100\%$$

i_2: Inhibition ratio of CuZn-SOD.

$$i_2 (\%) = \frac{(A_4-A_6)-(A_5-A_6)}{A_4-A_6} \times 100\% = \frac{A_4-A_5}{A_4-A_6} \times 100\%$$

A_1: The OD value of $T\text{-}SOD_{Control}$.

A_2: The OD value of $T\text{-}SOD_{Sample}$.

A_3: The OD value of $T\text{-}SOD_{Blank}$.

A_4: The OD value of $CuZn\text{-}SOD_{Control}$.

A_5: The OD value of $CuZn\text{-}SOD_{Sample}$.

A_6: The OD value of $CuZn\text{-}SOD_{Blank}$.

V_1: The total volume of the reaction system (mL).

V_2: The volume of sample added to the reaction system (mL).

f: Dilution factor of sample before test..

C_{pr}: Concentration of protein in sample, gprot/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	2.60	29.50	48.20
%CV	5.6	4.9	4.8

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	2.60	29.50	48.20
%CV	10.2	9.2	9.4

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/mL)	13	34	55
Observed Conc. (U/mL)	13.1	33.3	53.9
Recovery rate (%)	101	98	98

Sensitivity

The analytical sensitivity of the assay is 1.35 U/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix II Example Analysis

Example analysis：

The detection of T-SOD: Take 10% mouse liver tissue homogenate, dilute for 150 times with normal saline (0.9% NaCl), then take 5 μL of diluted sample, and carry the assay according to the operation steps. The results are as follows:

The average OD value of $T\text{-}SOD_{Sample}$ is 0.344, the average OD value of $T\text{-}SOD_{Control}$ is 0.546, the average OD value of $T\text{-}SOD_{Blank}$ is 0.121, the concentration of protein in 10% mouse liver tissue homogenate is 13.72 mgprot/mL, and the calculation result is:

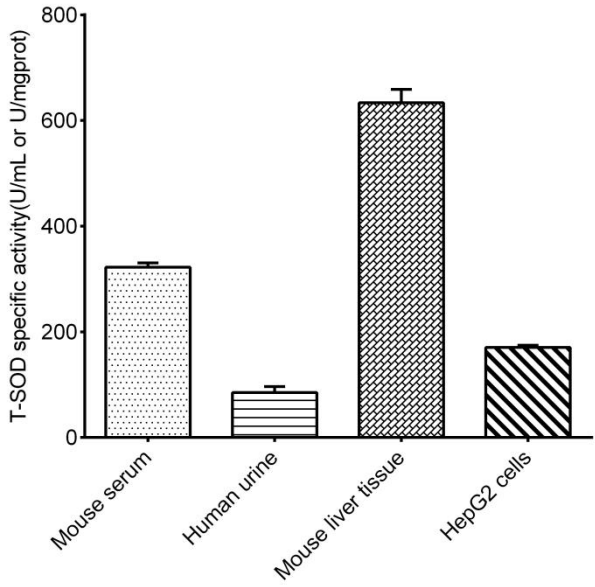
$$\text{T-SOD activity (U/mgprot)} = \left(\frac{0.546-0.344}{0.546-0.121}\right) \div 50\% \times \frac{0.305}{0.005} \times 150 \div 13.72 = 633.96 \text{ U/mgprot}$$

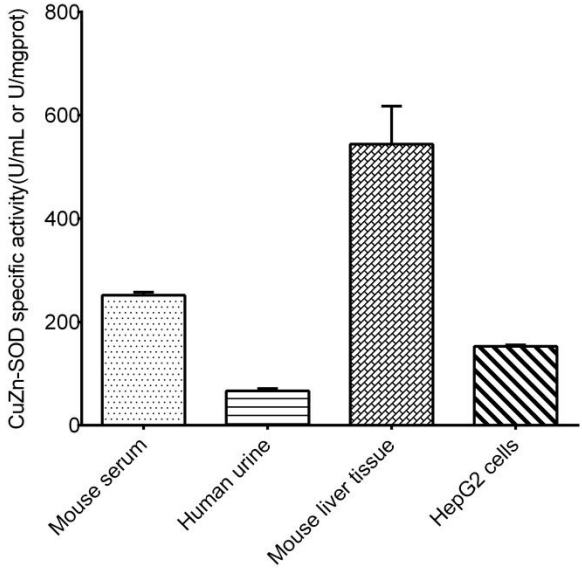
The detection of CuZn-SOD: pretreat the diluted sample with extracting solution, and carry the assay according to the operation steps. The results are as follows:

The average OD value of $CuZn\text{-}SOD_{Sample}$ is 0.400, the average OD value of $CuZn\text{-}SOD_{Control}$ is 0.590, the average OD value of $CuZn\text{-}SOD_{Blank}$ is 0.124, the concentration of protein in 10% mouse liver tissue homogenate is 13.72 mgprot/mL, and the calculation result is:

$$\text{CuZn-SOD activity (U/mgprot)} = \left(\frac{0.590-0.400}{0.590-0.124}\right) \div 50\% \times \frac{0.305}{0.005} \times 150 \div 13.72 = 543.83 \text{U/mgprot}$$

Detect mouse serum (dilute for 5 times), human urine (dilute for 2 times), 10% mouse liver tissue homogenate (the concentration of protein is 13.72 mgprot/mL, dilute for 150 times) and HepG2 cells (the concentration of protein is 5.21 mgprot/mL, dilute for 20 times) according to the protocol, the result is as follows：


T-SOD specific activity(U/mL or U/mgprot)
800
600
400
200
0
Mouse serum
Human urine
Mouse liver tissue
HepG2 cells


CuZn-SOD specific activity(U/mL or U/mgprot)
800
600
400
200
0
Mouse serum
Human urine
Mouse liver tissue
HepG2 cells

Appendix III Publications

1. Li X, Chen J, Feng W, et al. Berberine ameliorates iron levels and ferroptosis in the brain of 3× Tg-AD mice[J]. Phytomedicine, 2023, 118: 154962.

2. Tung Y T, Wu C H, Chen W C, et al. Ascophyllum nodosum and Fucus vesiculosus Extracts Improved Lipid Metabolism and Inflammation in High-Energy Diet–Induced Hyperlipidemia Rats[J]. Nutrients, 2022, 14(21): 4665.

3. Chen R, Gao S, Guan H, et al. Naringin protects human nucleus pulposus cells against TNF-α-induced inflammation, oxidative stress, and loss of cellular homeostasis by enhancing autophagic flux via AMPK/SIRT1 activation[J]. Oxidative Medicine and Cellular Longevity, 2022, 2022(1): 7655142.

4. Wang Y, Chi H, Xu F, et al. Cadmium chloride-induced apoptosis of HK-2 cells via interfering with mitochondrial respiratory chain[J]. Ecotoxicology and Environmental Safety, 2022, 236: 113494.

5. Yang L N, Xu S, Tang M, et al. The circadian rhythm gene Bmal1 ameliorates acute deoxynivalenol-induced liver damage[J]. Archives of Toxicology, 2023, 97(3): 787-804.

6. Lv D, Ji Y, Zhang Q, et al. Mailuoshutong pill for varicocele-associated male infertility—Phytochemical characterisation and multitarget mechanism[J]. Frontiers in Pharmacology, 2022, 13: 961011.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.